A ^{18}F Radiolabelled Zn(II) Sensing Fluorescent Probe

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Experimental Section

General Information

All chemical reagents were obtained from Sigma-Aldrich (Dorset, UK) and used without further purification. Solvents were purchased from VWR (Leicestershire, UK). Metal ion solutions were prepared from metal (II) chlorides by addition of 10 mM HEPES (pH 7.68). HPLC water was used throughout and was purchased from VWR (Leicestershire, UK). Absorption spectra were collected on a ThermoFisher Scientific Evolution 300 UV-Vis spectrophotometer. Fluorescence spectra were collected on a Horiba Fluoromax-4P spectrofluorometer. Metal titrations were performed by sequential addition of aliquots of the metal ion solution to a sample of 0.1 mM AQA-F in 10 mM HEPES (pH 7.68). The concentrations of metal ions used in these titrations were 0, 2.5, 5, 7.5, 10, 12.5, 15, 17.5, 20, 22.5, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70 ,75, 80 , 85, 90, 95, 100, 125, 150, 175, 200, 250, 300 µM. Cellular imaging was taken using an ImageXpress Micro 4 high throughput fluorescence microscope. Micrographs were then analysed using MetaXpress and ImageJ software. Emission intensities *in vitro* were calculated using an area of 578912 pixels on ImageJ and were reported as an average of 3. NMR spectra were obtained on a JEOL JNM-LA400 Spectrometer. FTIR spectra was taken on Perkin Elmer Rx FTIR x2 with diamond ATR, DRIFT attachment. Mass spectrometry data was collected using an Advion MS SOP electrospray ionisation (ESI) spectrometer. pH was measured using a Jenway 3520 digital pH meter with a Mettler-Toledo 51343160 glass electrode.

Data Fitting

Dissociation constants were determined by fitting absorption data with a one site total binding model using Graphpad Prism 6.05. pKa values were determined by fitting fluorescence data with an asymmetric sigmoidal 5PL model using Graphpad Prism 6.05. Absorption and Fluorescence data were subsequently smoothed using the Savitzky-Golay method with a polynomial degree of 2 on Matlab.

***In Vitro* Studies**

RWPE-1 cells were cultured in Keratinocyte Serum Free Medium (K-SFM, Fisher Scientific, Loughborough, UK) supplemented with 50 mg/L bovine pituitary extract (Fisher Scientific, Loughborough, UK) and 5 µg/L human recombinant epidermal growth factor (Fisher Scientific, Loughborough, UK) at 37 ^{o}C under 5% CO_2 atmosphere.

PC-3 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM, Lonza, Castleford, UK) supplemented with 10% foetal bovine serum (Lonza, Castleford, UK) and 1% PenStrep (Lonza, Castleford, UK) at 37 ^{o}C under 5% CO_2 atmosphere.

Cells were cultured and seeded into a falcon 96-well plate at a cell density of $1x10^5$ cells/ml. The cells were then incubated overnight at 37 ^{o}C in 200 µl of corresponding media. The cells were then incubated at 37 ^{o}C for 30 minutes in Krebs buffer with various dye combinations. **AQA-F** was incubated at concentrations of 100 µM and Rhodamine Concanavalin A. After the incubation period, the treatments were removed, the cells were washed twice with phosphate buffered saline and fixed with 4% paraformaldehyde.

High Performance Liquid Chromatography (HPLC)

Analytical*: Column*: Agilent Zorbax Eclipse XDB-C18 4.6 x 150 mm, 5-micron. *Gradient:* Solvent A: Water + 0.1% TFA, Solvent B: Acetonitrile + 0.1% TFA.

Table S 1: HPLC gradient used for analytical HPLC

Time / mins	Solvent A / %	Solvent B / %
0	95	5
3	95	5
18	5	95
20	5	95
25	95	5
30	95	5

Semi-Preparative: Column: Agilent Zorbax Eclipse XDB-C18, 9.4 x 250 mm, 5 micron. *Gradient*: Solvent A: Water + 0.1% TFA, Solvent B: Acetonitrile + 0.1% TFA.

Table S 2: HPLC gradient used for semi-preparative HPLC

Time / mins	Solvent A / %	Solvent B / %
0	95	5
5	95	5
20	50	50
21	5	95
24	5	95
25	95	5
28	95	5

Ligand Synthesis

Synthesis of 2-chloro-N-(quinolin-8-yl)acetamide (**2**)

2-chloroacetyl chloride (1.3 mL, 16.6 mmol) dissolved in chloroform (30 mL) was added dropwise to a cooled solution of 8-aminoquinoline (**1**) (2.056 g, 14.3 mmol), pyridine (1.6 mL, 19.4 mmol) and chloroform (90 mL) over a period of 1 hour. After stirring for 2 hours, the solvent was removed under reduced pressure. The resulting product was dissolved in DCM (50 mL), extracted with water (3 x 50 mL) and dried over $MgSO_4$ to yield crude product as an oil. The crude product was then purified by column chromatography (silica, DCM) to yield an off white solid **(2)** (1.405 g, 6.4 mmol, 45%).

^{1}H NMR [400 MHz, $CDCl_3$, 289 K] δ: 10.91 (s, 1 H; ~N***H***~), 8.87 (dd, $^3J_{HH}$ = 4.2 Hz, $^4J_{HH}$ = 1.7 Hz, 1H; ~NHCCHCHC***H***~), 8.76 (dd, $^3J_{HH}$ = 6.4, 2.6 Hz, 1H; ~NCHCHC***H***~), 8.18 (dd, $^3J_{HH}$ = 8.3 Hz, $^4J_{HH}$ = 1.7 Hz, 1H; ~NHCC***H***CHCH~), 7.59 (m, 2 H; ~NHCCHC***H***CH~ ~NCHC***H***CH~), 7.49 (q, $^3J_{HH}$ = 4.2 Hz, 1H; ~NC***H***CHCH~), 4.32 (s, 2 H; ~NC(=O)C***H$_2$***Cl). ^{13}C NMR [100 MHz, $CDCl_3$, 289 K] δ: 164.48 (**C**=O), 148.79 (~NH***C***CHCHCH~), 136.39 (~N***C***HCHCH~), 128.05 (~NHCCH***C***HCH~), 127.27 (~NCH***C***HCH~), 122.65 (~NHCCHCH***C***H~), 121.91 (~NCHCH***C***H~), 116.74 (~NHC***C***HCHCH~), 43.45 (~***C***H$_2$Cl). FTIR: $\bar{\nu}$= 3322 (NH stretch), 1668 (C=O), 1589 (NH bend), 1328 (C=N), 826 cm^{-1} (C-Cl). MS (ESI) m/z: 220.6 (75%, [*M*(^{35}Cl)+H]$^+$), 222.6 (25%, [*M*(^{37}Cl)+H]$^+$).

Synthesis of tert-butyl (2-hydroxyethyl)carbamate (**3**)

Ethanolamine (9.9 mL, 81.9 mmol), triethylamine (27.4 mL, 98.3 mmol), di-*tert*-butyl dicarbonate (21.375 g, 98.4 mmol) in DCM (250 mL) were stirred for 12 hours. The reaction mixture was then concentrated under reduced pressure and was extracted using EtOAc (50 mL) and water (50 mL). The organic layer was collected, dried over sodium sulfate and then concentrated under reduced pressure to give a colourless oil **(3)** (10.008 g, 62.0 mmol, 75%).

^{1}H NMR [400 MHz, $CDCl_3$, 289 K] δ: 5.04 (s, 1 H; OHCH$_2$CH$_2$N***H***~), 3.67 (t, 2 H; OHCH$_2$C***H$_2$***NH~), 3.26 (d, $^2J_{HH}$ = 4.9 Hz, 2H; OHC***H$_2$***CH$_2$NH~), 2.82 (s, 1H; ~O***H***), 1.44 (s, 9H; ~OC(C***H$_3$***)$_3$~). ^{13}C NMR [100 MHz, $CDCl_3$, 289 K] δ: 157.01 (***C***=O), 79.83 (~NHCH$_2$***C***H$_2$OH), 62.92 (~NH***C***H$_2$CH$_2$OH), 43.27 (~O***C***(CH$_3$)$_3$), 28.45 (~OC(***C***H$_3$)$_3$). FTIR: $\bar{\nu}$= 3325 (OH and NH stretch), 1689 (C=O) 1525 (N-H bending), 1071 (C-O). MS (ESI): 183.6 [*M*+Na]$^+$.

Synthesis of 2-fluoroethyl 4-methylbenzenesulfonate (**4**)

2-fluoroethanol (1.8 mL, 31.0 mmol) was dissolved in chloroform (30 mL) and cooled to 0 °C, pyridine (5.0 mL, 62.0 mmol) was added followed by the addition of *p*-toluenesulfonyl chloride (8.94 g, 46.9 mmol) dropwise. The reaction was stirred at 0 °C for 2.5 hours. The chloroform was removed under reduced pressure and the resulting solution was taken up in DCM (100 mL) and washed with 1 M HCl (60 mL) and then with 1 M K_2CO_3 (100 mL). The organic layer was dried over magnesium sulfate and the solvent was removed. The crude product was purified by column chromatography (silica, 20% EtOAc/80% hexane) to yield a colourless oil **(4)** (5.772 g, 26.4 mmol, 85%).

^{1}H NMR [400 MHz, $CDCl_3$, 289 K] δ: 7.81 (d, $^4J_{HH}$ = 8.3 Hz, 2H; CH$_3$CCHC***H***CC***H***CH~), 7.36 (d, $^4J_{HH}$ = 7.5 Hz, 2H, CH$_3$CC***H***CHCCHC***H***), 4.57 (dm, 2H; ~OCH$_2$C***H$_2$***F~), 4.26 (dm, 2H; ~OC***H$_2$***CH$_2$F~), 2.45 (s, 3H; C***H$_3$***~). ^{13}C NMR [100 MHz, $CDCl_3$, 289 K] δ: 145.24 (CH$_3$CCHCH***C***SO$_3$~), 132.73 (CH$_3$***C***HCHCSO$_3$~), 130.03 (CH$_3$CCH***C***HCSO$_3$~), 128.08 (CH$_3$C***C***HCHCSO$_3$~), 80.63 (d, ~OCH$_2$***C***H$_2$F, $^1J_{CF}$ = 173.8 Hz), 68.53 (d, ~O***C***H$_2$CH$_2$F, $^2J_{CF}$ = 21 Hz), 21.76 (~***C***H$_3$). ^{19}F NMR [376 MHz, $CDCl_3$, 289 K] δ: -224.51 (tt, $^2J_{HF}$ = 47 Hz, $^3J_{HF}$ = 27 Hz). FTIR: $\bar{\nu}$= 1458 (C-F). MS (ESI): 240.5 [*M*+Na]$^+$.

Synthesis of tert-butyl ((2-fluoroethoxy)methyl)carbamate (**5**)

NaH (0.330 g, 13.8 mol) was added in portions to a solution of **3** (2.4 mL, 14.0 mmol) and **4** (3.000 g, 13.3 mmol) in anhydrous DMF (30 mL). The reaction was left to stir overnight under argon. After 18 hours, the reaction mixture was diluted with EtOAc (30 mL) and water (30 mL) was slowly added. The organic phase was collected and dried using sodium sulfate. The resultant solution was then concentrated under reduced pressure to yield a crude oil, which was purified *via* column chromatography (silica, 10% EtOAc/90% hexane – 40% EtOAc/60% hexane) to yield a colourless oil **(5)** (0.602 g, 2.9 mmol, 22%).

^{1}H NMR [400 MHz, $CDCl_3$, 289 K] δ: 4.89 (s, 1H; ~N***H***~), 4.60-4.48 (m, 2H; FC***H$_2$***CH$_2$O~), 3.65 (m, 2H; ~OC***H$_2$***CH$_2$NH~), 3.56 (t, $^3J_{HF}$ = 5.1 Hz, 2H; FCH$_2$C***H$_2$***O~), 3.32 (dd, *J* = 10.5, 5.3 Hz, 2H; ~NHC***H$_2$***CH$_2$O~), 1.43 (s, 9H; ~OC(C***H$_3$***)$_3$). ^{13}C NMR [100 MHz, $CDCl_3$, 289 K] δ: 156.05 (***C***=O), 83.07 (d, $^1J_{CF}$ = 169 Hz), 79.41, 70.54, 70.18 (d, $^2J_{CF}$ = 19.6 Hz), 28.47 (~OC(***C***H$_3$)$_3$). ^{19}F NMR [376 MHz, $CDCl_3$, 289 K] δ: -222.99 (tt, $^2J_{HF}$ = 47.5 Hz, $^3J_{HF}$ = 29 Hz). FTIR: $\bar{\nu}$= 1695 (NH scondary amide stretch), 1455 (C-F), 1168 (C-O), 1129 (C-O). MS (ESI): 229.7 [*M*+Na]$^+$, 245.6 [*M*+K]$^+$.

Synthesis of (2-fluoroethoxy)methanamine hydrochloride (**6**)

To a solution of **5** (0.602 g, 3.1 mmol) in DCM (4 mL), TFA (4 mL) was added. After 1 hour of stirring, the reaction mixture was concentrated under reduced pressure to yield a crude oil. The crude product precipitated as an amine hydrochloride salt upon addition of 1 M solution of HCl in diethyl ether (6 mL), which was filtered and obtained as a white powder (**6**) (0.382 g, 2.9 mmol, 94%).

^{1}H NMR [400 MHz, $CDCl_3$, 289 K] δ: 8.38 (s, 2H; N***H₂***~), 4.72 – 4.49 (m, 2H; ~OCH$_2$C***H₂***F), 3.90 – 3.72 (m, 4H; NH$_2$CH$_2$C***H₂***OC***H₂***~), 3.23 (t, 2H; NH$_2$C***H₂***CH$_2$O~). ^{13}C NMR [100 MHz, $CDCl_3$, 289 K] δ: 77.42, 77.30, 76.93, 76.78. ^{19}F NMR [376 MHz, $CDCl_3$, 289 K] δ: -222.71, (tt, $^2J_{HF}$ = 47.7 Hz, $^3J_{HF}$ = 30.3 Hz). FTIR: $\tilde{\nu}$= 2890 (N-H salt stretch), 1456 (C-F), 1120 (C-O). MS (ESI): 108.2 [*M*+H]$^+$.

Synthesis of 2-((2-(2-fluoroethoxy)ethyl)amino)-N-(quinolin-8-yl)acetamide (**AQA-F**)

6 (0.350 g, 2.7 mmol), **2** (0.108 g, 0.5 mmol), DIPEA (1.3 mL, 7.3 mmol) and a catalytic amount of KI were heated together under reflux in acetonitrile (30 mL). After 18 hours, the reaction was cooled to room temperature and concentrated under reduced pressure to yield an orange oil. The crude product was purified *via* column chromatography (silica, DCM/1% MeOH) to yield an orange oil, **AQA-F** (0.123 g, 0.4 mmol, 80%).

^{1}H NMR [400 MHz, $CDCl_3$, 289 K] δ: 9.00 (dd, 1 H, ***Ar***, $^3J_{HH}$ = 4.6 Hz, $^4J_{HH}$ = 1.4 Hz), 8.43 (dd, ***Ar***, 1 H, $^3J_{HH}$ = 8.3 Hz, $^4J_{HH}$ = 0.9 Hz), 7.68 (dd, 1 H, ***Ar***, $^3J_{HH}$ = 8.3 Hz, $^4J_{HH}$ = 0.9 Hz), 7.62 (dd, 1 H, ***Ar***, $^3J_{HH}$ = 8.3 Hz, $^4J_{HH}$ = 4.6 Hz), 7.58 (t, 1 H, ***Ar***, $^3J_{HH}$ = 8.3 Hz), 4.57 (dt, 2 H, -OCH$_2$C***H₂***F, $^2J_{HF}$ = 47.7 Hz, $^3J_{HH}$ = 4.1 Hz), 4.34 (s, 2 H, ArNHC(O)C***H₂***N-), 3.94 (t, 2 H, -NCH$_2$C***H₂***O-, $^3J_{HH}$ = 4.6 Hz), 3.78 (dt, 2 H, -OC***H₂***CH$_2$F, $^3J_{HF}$ = 30.3 Hz, $^3J_{HH}$ = 4.1 Hz), 3.48 (t, 2 H, -NCH$_2$C***H₂***O-, $^3J_{HH}$ = 4.6 Hz). ^{19}F NMR [376 MHz, $CDCl_3$, 298 K] δ: -225.74 (tt, -OCH$_2$CH$_2$***F***, $^2J_{HF}$ = 47.7 Hz, $^3J_{HF}$ = 30.3 Hz). ^{13}C NMR [100 MHz, $CDCl_3$, 289 K] δ: 164.01 (s, ArNH***C***(O)CH$_2$NH-), 147.40 (s, ***Ar***), 139.77 (s, ***Ar***), 136.54 (s, ***Ar***), 131.47 (s, ***Ar***), 128.43 (s, ***Ar***), 128.16 (s, ***Ar***), 124.28 (s, ***Ar***), 121.80 (s, ***Ar***), 82.86 (d, -OCH$_2$***C***H$_2$F, $^1J_{HF}$ = 170.5 Hz), 70.39 (d, -O***C***H$_2$CH$_2$F, $^2J_{CF}$ = 19.3 Hz), 66.27 (s, -NCH$_2$***C***H$_2$O-), 49.66 (s, ArNHC(O)***C***H$_2$N-), 47.92 (s, -N***C***H$_2$CH$_2$O-). FTIR: $\tilde{\nu}$= 1683 (C=O), 1324 (aromatic amine stretch), 1595 (N-H bending), 1456 (C-F), 1126 (C-O). MS (ESI): 292.1 [M+H]$^+$, 314.2 [M+Na]$^+$.

Synthesis of tert-butyl (2-(2-hydroxyethoxy)ethyl)(2-oxo-2-(quinolin-8-ylamino)ethyl)carbamate (**7**)

AQZ (590 mg, 2.04 mmol), Boc_2O (534 mg, 2.45 mmol), Et_3N (0.28 ml, 2.04 mmol) were stirred in DCM (60 ml) for 12 hours. The reaction was monitored via TLC, and concentrated under reduced pressure and purified via column chromatography (DCM-MeOH) as a yellow solid (715 mg, 1.84 mmol, 90%).

^{1}H NMR [400 MHz, $CDCl_3$, 289 K] δ: = 10.37 (1 H, br, N***H***); 8.70 (2 H, br, NHCC***H***CHCH, NC***H***CHCH); 8.20 (1 H, d, $^3J_{HH}$ = 7.7 Hz, NHCCHC***H***CH); 7.57-7.48 (3 H, m, NCHC***H***CH, NHCCHCHC***H***, NCHCHC***H***); 4.23 (2 H, m,); 3.77-3.46 (8 H, m, NC***H₂C H₂***O, OC***H₂C H₂***OH); 1.45 (9 H, s, (C***H₃***)$_3$). ^{13}C NMR [100 MHz, $CDCl_3$, 289 K] δ: = 169.0 (***C***O), 155.3 (***C***O), 148.4 (NH***C***CHCHCH), 138.5 (NHC***C***N), 136.3 (N***C***HCHCH), 134.1 (NHCC***C***CH), 128.0 (NHCCH***C***HCH), 127.4 (NCH***C***HCH), 121.9 (NHCCHCH***C***H), 121.7 (NCHCH***C***H), 116.6 (NHC***C***HCHCH), 81.3 (O***C***(CH$_3$)$_3$), 72.5 (CO***C***H$_2$NH), 70.3 (OCH$_2$***C***H$_2$OH), 61.7 (O***C***H$_2$CH$_2$OH), 54.0 (NHCH$_2$***C***H$_2$O), 48.5 (NH***C***H$_2$CH$_2$O), 28.2 ((***C***H$_3$)$_3$). IR, ν_{max}/cm^{-1}: 3326 (OH and NH stretch), 1682 (C=O), 1457 (CH$_3$), 1392 (CH$_3$), 1246 (C-O), 1165 (C-O), 1136 (C-O). MS(ESI+): *m/z* = 390 amu [M+H]$^+$; 412 amu [M+Na]$^+$.

Synthesis of 2-(2-((tert-butoxycarbonyl)(2-oxo-2-(quinolin-8-ylamino)ethyl)amino)ethoxy)ethyl 4-methylbenzenesulfonate (**8**)

7 (395 mg, 1.01 mmol) was dissolved in DCM (50 ml) and cooled to 0 ^{o}C. Triethylamine (0.42 ml, 3.04 mmol) and p-Toluenesulfonyl chloride (482 mg, 2.53 mmol) were then added dropwise over 30 minutes. Following the complete addition of the reagents, the reaction mixture was stirred for a further 10 minutes, and then at room temperature for 12 hours. The reaction was monitored via TLC, and purified via column chromatography (DCM-MeOH) to yield a yellow oil, (450 mg, 0.83 mmol, 82%).

^{1}H NMR [400 MHz, $CDCl_3$, 289 K] δ: 10.29 (m, 1 H, QN***H***C(=O)CH$_2$N-), 8.76 (br s, 2 H, Q), 8.17 (br d, 1 H, Q), 7.74-7.67 (m, 2 H, Ts), 7.53 (m, 2 H, Q), 7.47-7.44 (m, 1 H, Q), 7.32-7.27 (m, 2 H, Ts), 4.21-4.05 (m, 2 H, QNHC(=O)C***H₂***N-), 4.00-3.92 (m, 2 H, -C**H₂**-), 3.68 (m, 1 H, -C**H₂**-), 3.63-3.48 (m, 5 H, -C**H₂**-), 2.41 (s, 3 H, -OS(=O)$_2$PhC***H₃***), 1.54 (s, 3 H, C(C***H₃***)$_3$), 1.37 (C(C***H₃***)$_3$). ^{13}C NMR [100 MHz, $CDCl_3$, 289 K] δ: 168.36 (QNH***C***(=O)CH$_2$N-), 155.11 (N***C***(=O)OC(CH$_3$)$_3$), 145.24 (**Q**), 144.73 (**Ts**), 138.24 (**Q**), 136.26 (**Q**), 134.01 (**Ts**), 132.72 (**Q**), 129.72 (**Ts**), 127.88 (**Ts**, **Q**), 127.79 (**Q**), 127.26 (**Q**), 121.66 (**Q**), 116.27 (**Q**), 80.99 (-O***C***(CH$_3$)$_3$), 70.45 (-**C**H$_2$-), 68.94 (-**C**H$_2$-), 68.31 (-**C**H$_2$-), 54.49 (-N***C***H$_2$CH$_2$O-), 53.49, 48.63 (-**C**H$_2$-), 28.34 (C(***C***H$_3$)$_3$), 29.04 (C(***C***H$_3$)$_3$), 21.58 (-OS(=O)$_2$Ph***C***H$_3$) MS(ESI+): *m/z* = 544 amu [M+H]$^+$

Synthesis of [^{18}F]**AQA-F**

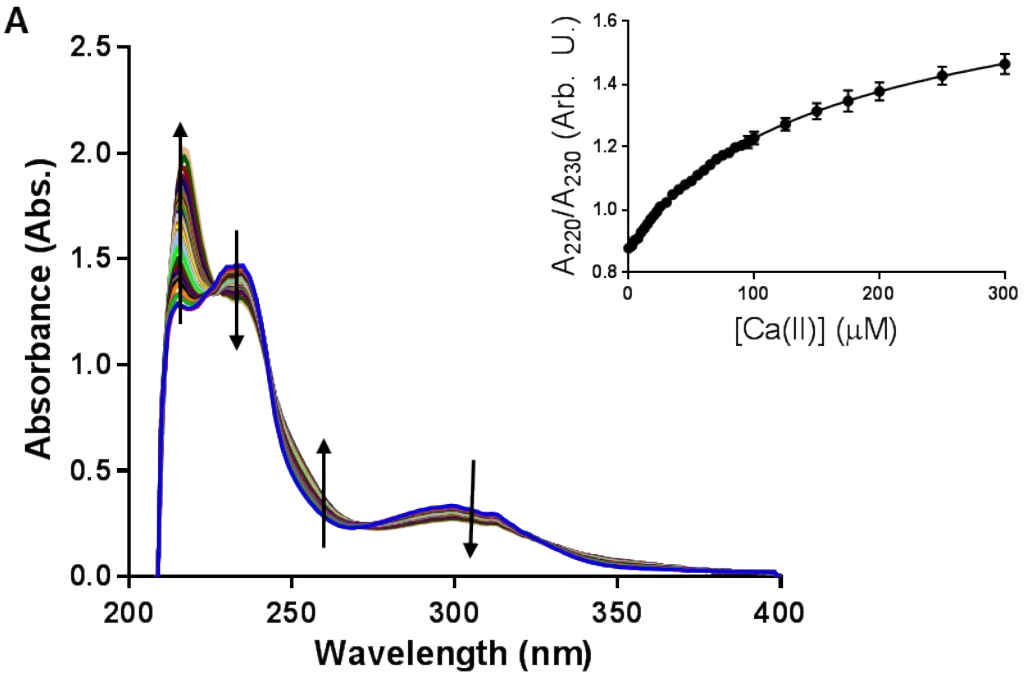
[^{18}F]F$^-$ (~1 GBq) produced by cyclotron (GE PETtrace 6 cyclotron) bombardment of ^{18}O enriched water using the ^{18}O(p,n)^{18}F reaction was trapped on a Sep-Pak Light QMA cartridge (pre-activated by passing 8.4% $NaHCO_3$ solution (5 mL), water (20 mL) and air (40 mL) through the cartridge). The cartridge was dried with air and the activity eluted with K_2CO_3 solution (0.5

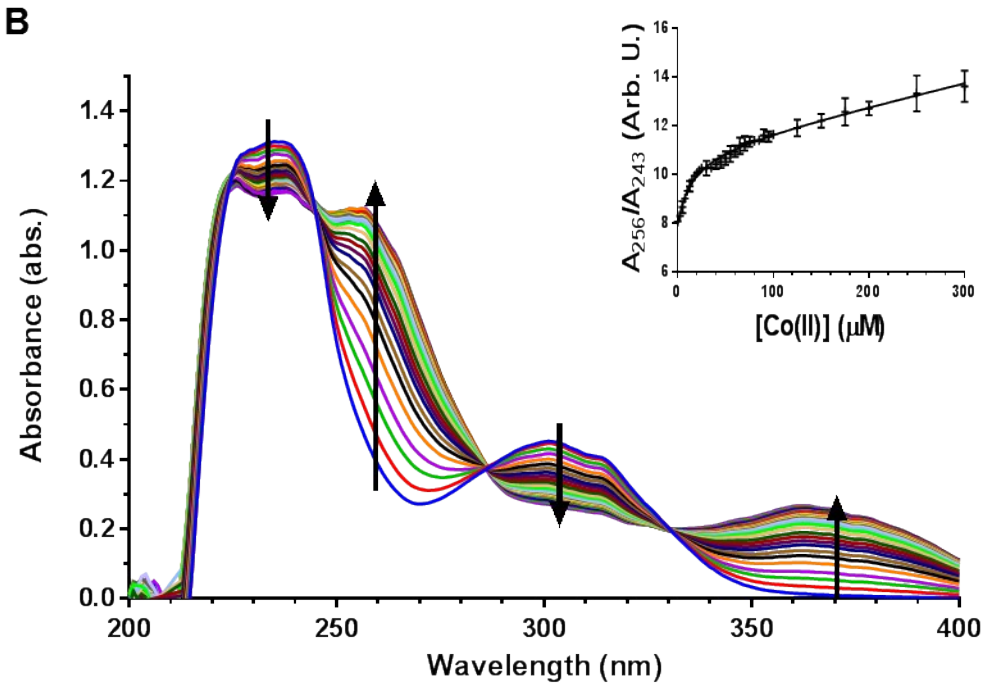
mL, 3.6 mg mL^{-1}) into a vial containing Kryptofix (10 mg) and anhydrous acetonitrile (1 mL). The solution was dried at 110 °C under a stream of argon and further dried by addition of anhydrous acetonitrile (3 x 1 mL).

To the dried $[^{18}F]F^-$ (743 MBq) was added a solution of **8** (6 mg) in acetonitrile (300 µL). The solution was heated to 110 °C for 15 minutes in a sealed container. TFA (300 µL) was added and heating continued for 15 minutes. Water (300 µL) was added and the reaction mixture purified by semi-preparative HPLC. The fraction 16:45 – 17:45 minutes was collected to give $[^{18}F]$**AQA-F** in an isolated after HPLC purification radiochemical yield of 8.6% and a radiochemical purity of 97% with a total synthesis time of 88 minutes after the end of bombardment.

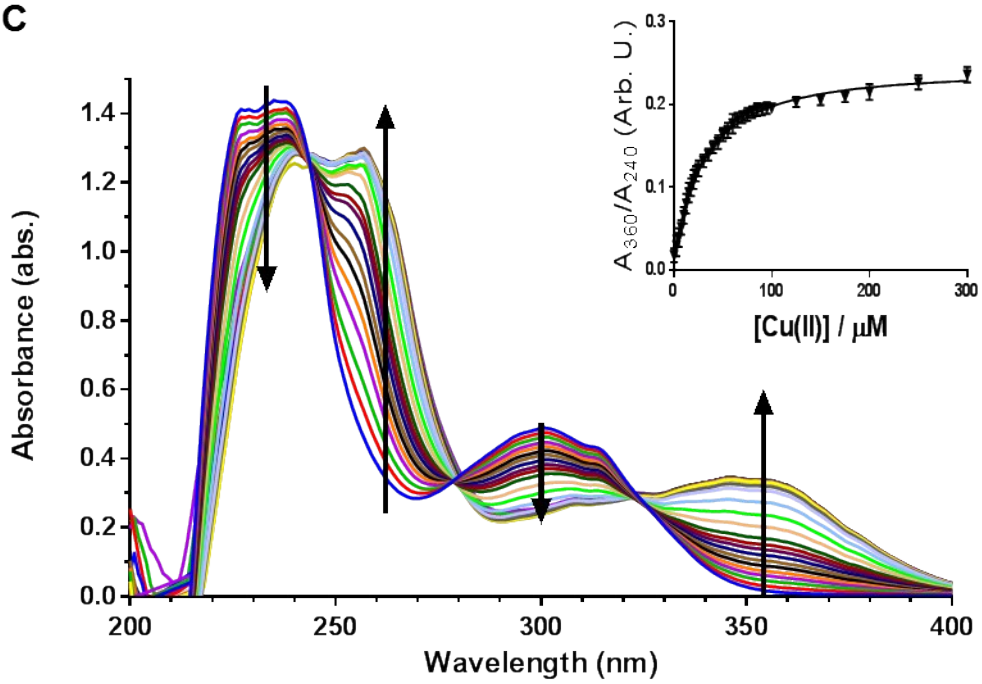
Table S 3: Dissociation constants for **AQA-F** with divalent metal ions determined by metal titration (n = 3).

Metal Ion	Dissociation constant, K_d (µM)
Ca^{2+}	123.6 ± 19.1
Co^{2+}	15.4 ± 2.9
Cu^{2+}	30.4 ± 3.0
Fe^{2+}	65.7 ± 17.5
Hg^{2+}	24.3 ± 2.9
Mn^{2+}	55.9 ± 10.3
Zn^{2+}	15.2 ± 1.6

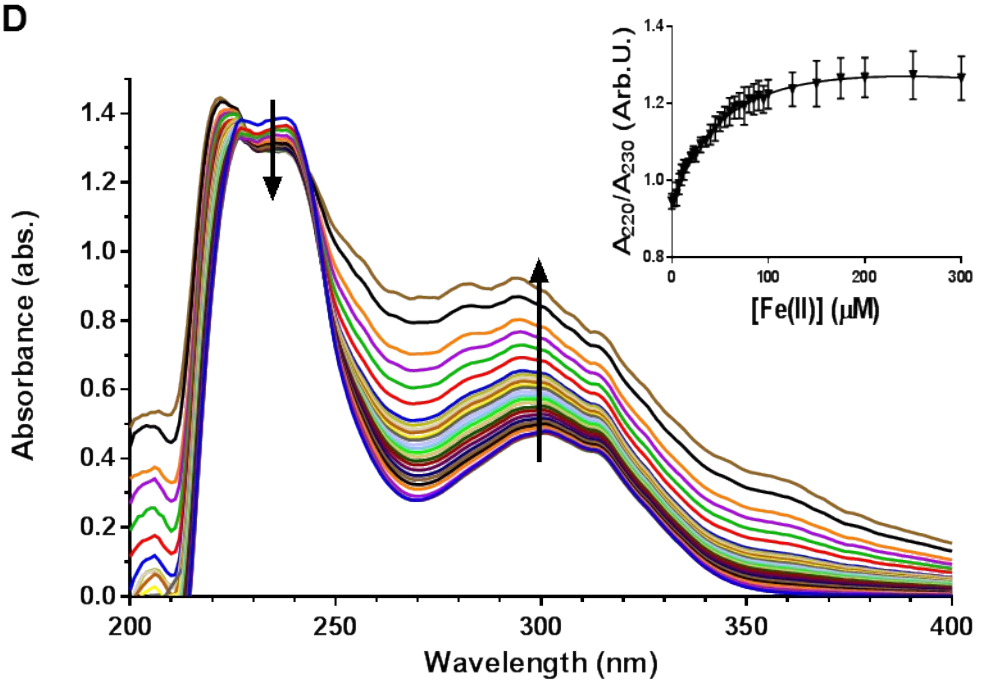

A
Absorbance (Abs.)
Wavelength (nm)
A220/A230 (Arb. U.)
[Ca(II)] (μM)


B
Absorbance (abs.)
Wavelength (nm)
A256/A243 (Arb. U.)
[Co(II)] (μM)

C


Absorbance (abs.)
Wavelength (nm)
A360/A240 (Arb. U.)
[Cu(II)] / μM

D


Absorbance (abs.)
Wavelength (nm)
A220/A230 (Arb.U.)
[Fe(II)] (μM)

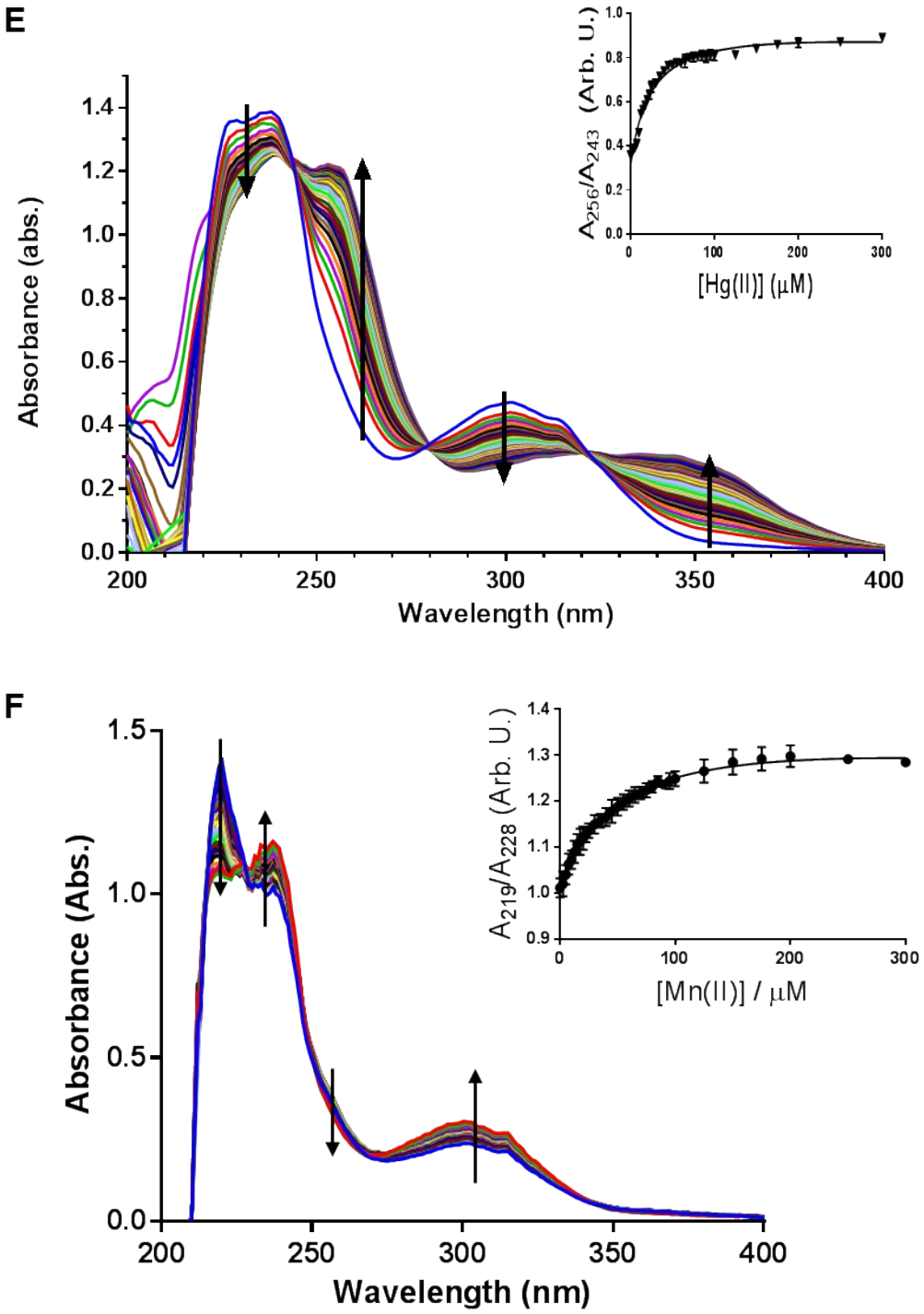


Figure S 1: Absorption spectra of **AQA-F** (0.1 mM) in HEPES buffer (10 mM, pH = 7.68) in the presence of increasing concentrations of metal chloride (0-3 equiv). (Inset:) Ratiometric curve A_{350}/A_{300} nm as a function of metal concentration. A = Ca(II), B = Co(II), C = Cu(II), D = Fe(II), E = Hg(II), F = Mn(II) (n = 3).

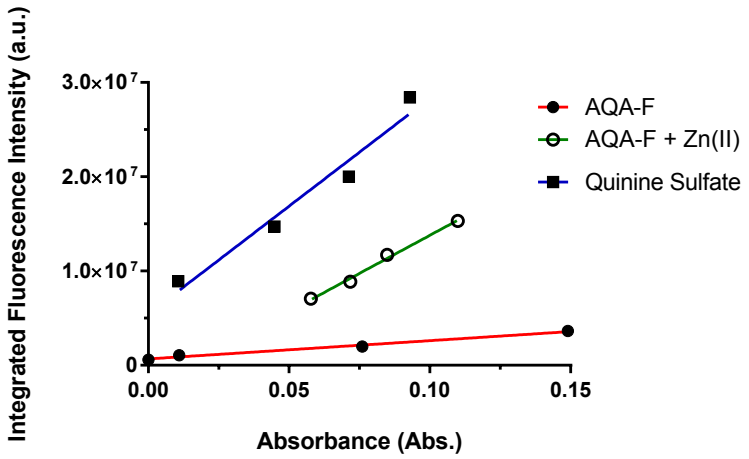


Figure S 2: Plot of absorbance against integrated fluorescence intensity for **AQA-F**, **AQA-F** + 1 equivalent Zn(II)Cl_2, and quinine sulfate (λ_{ex}=320 nm).

Table S 4*:* Quantum yields for **AQA-F** in the absence and presence of one equivalent of $ZnCl_2$ compared to quinine sulfate as standard.

Compound	Gradient of linear regression	Φ_f
AQA-F	1.938×10^7	0.042 (4.2%)
AQA-F + $ZnCl_2$	1.617×10^8	0.35 (35%)
Quinine Sulfate	2.289×10^8	0.5 (50%)

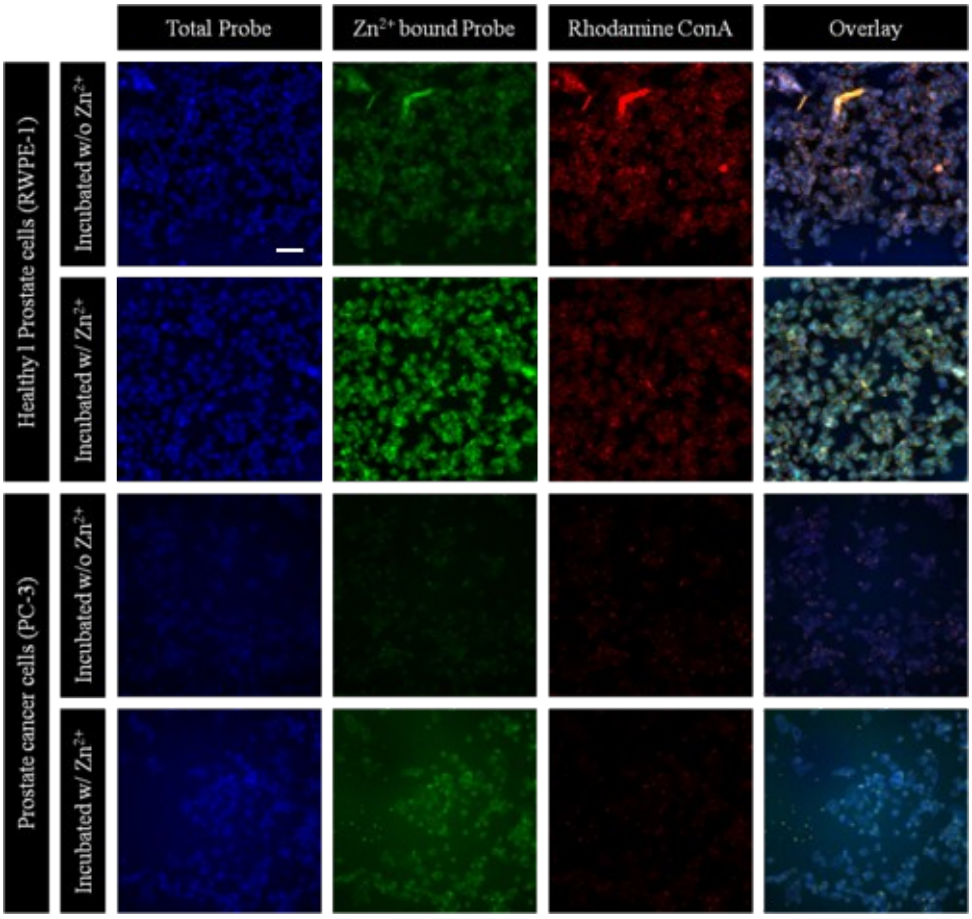


Figure S 3: A collection of micrographs showing RWPE-1 and PC-3 cells that have been incubated with 100 µM **AQA-F** and Rhodamine Concanavalin A. Scale bar = 50 µM. (n = 3)

Table S 5: Emission intensity of **AQA-F** (100 µM) when incubated for 30 minutes in RWPE-1 and PC-3 cells in the presence

Cell Line	Sample	Blue emission			Green Emission			Ratio emission intensity Green/Blue
		Mean	Min	Max	Mean	Min	Max	
RWPE-1	Without Zn(II)	58.677	0	255	33.847	0	185	0.58
	With Zn(II)	62.725	0	255	59.462	0	255	0.95
PC-3	Without Zn(II)	15.670	8	43	5.223	2	34	0.33
	With Zn(II)	30.729	18	74	19.079	9	82	0.62

and absence of a $ZnCl_2$ solution (n = 3).

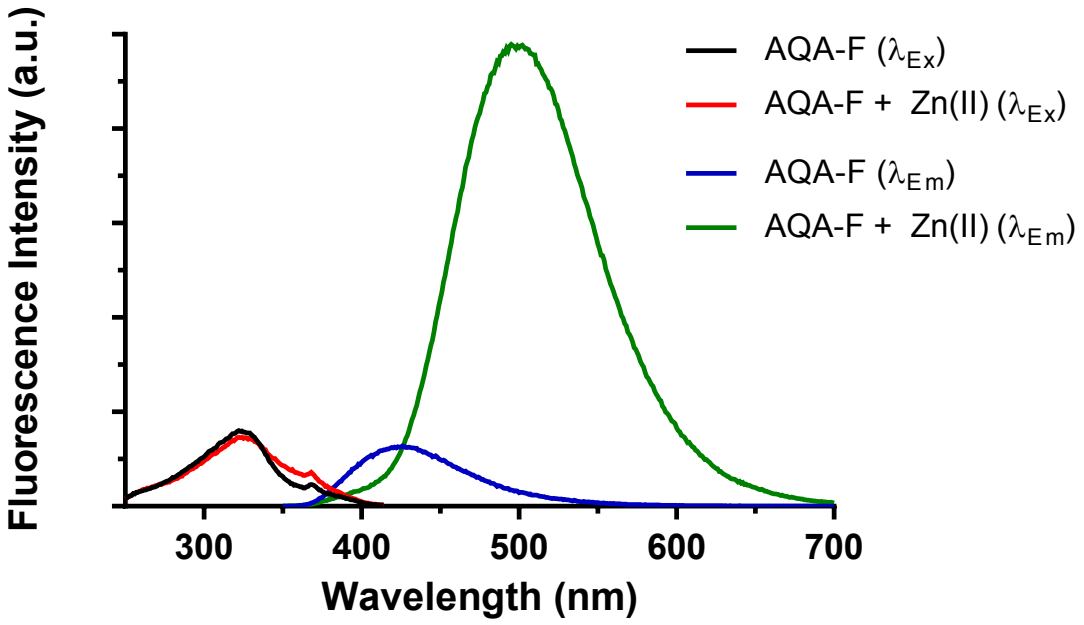


Figure S 4: Excitation and emission spectra for **AQA-F** (0.1 mM) in HEPES buffer (10 mM, pH = 7.68) in the absence and presence of one equivalent of $Zn(II)Cl_2$.

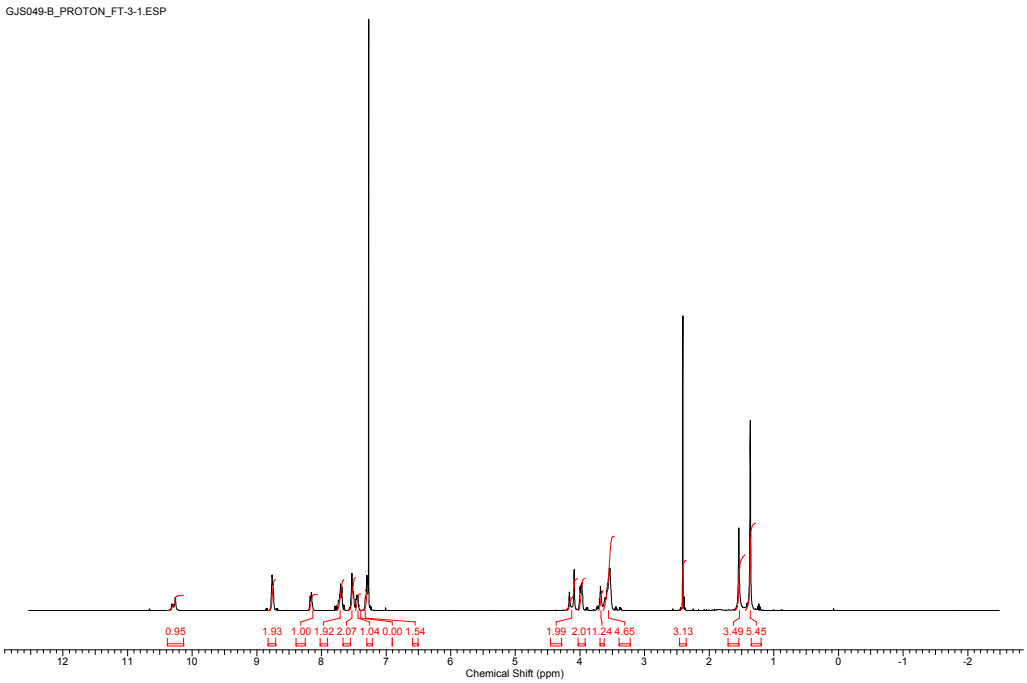


Figure S 5: ^{1}H NMR of **8** (5 mg ml^{-1}).

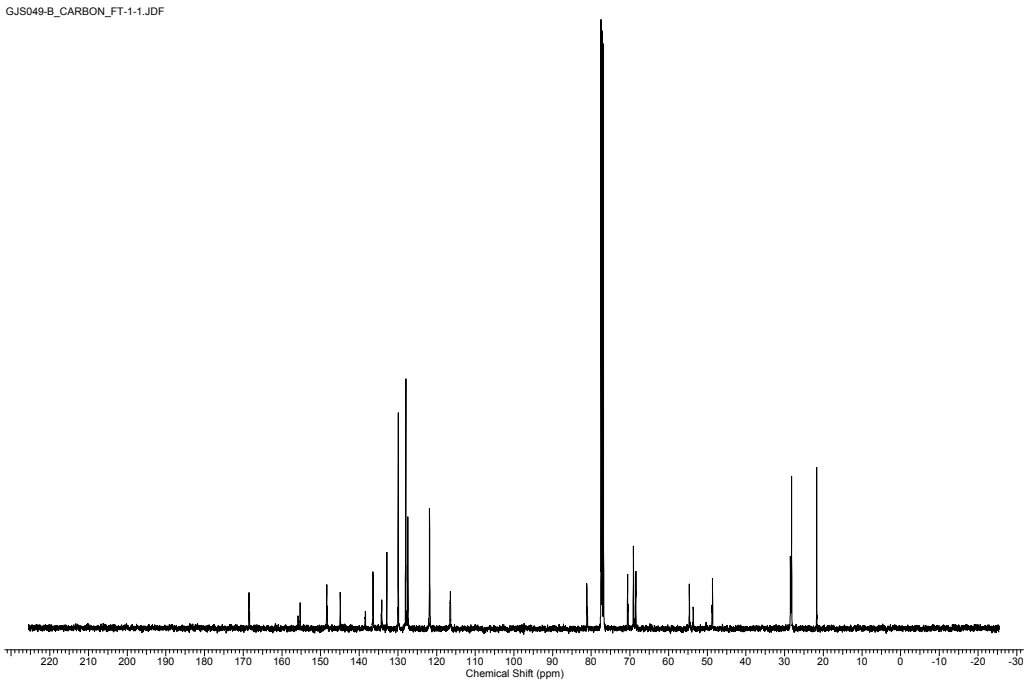


Figure S 6: ^{13}C NMR of **8** (5 mg ml^{-1})

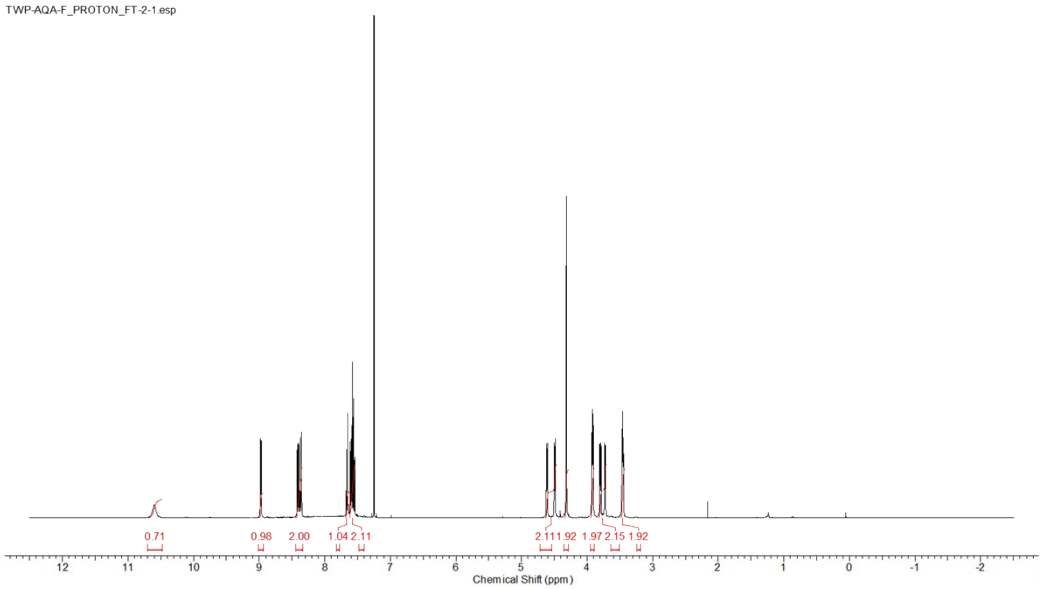


Figure S 7: ^{1}H NMR of **AQA-F** (5 mg ml^{-1}).

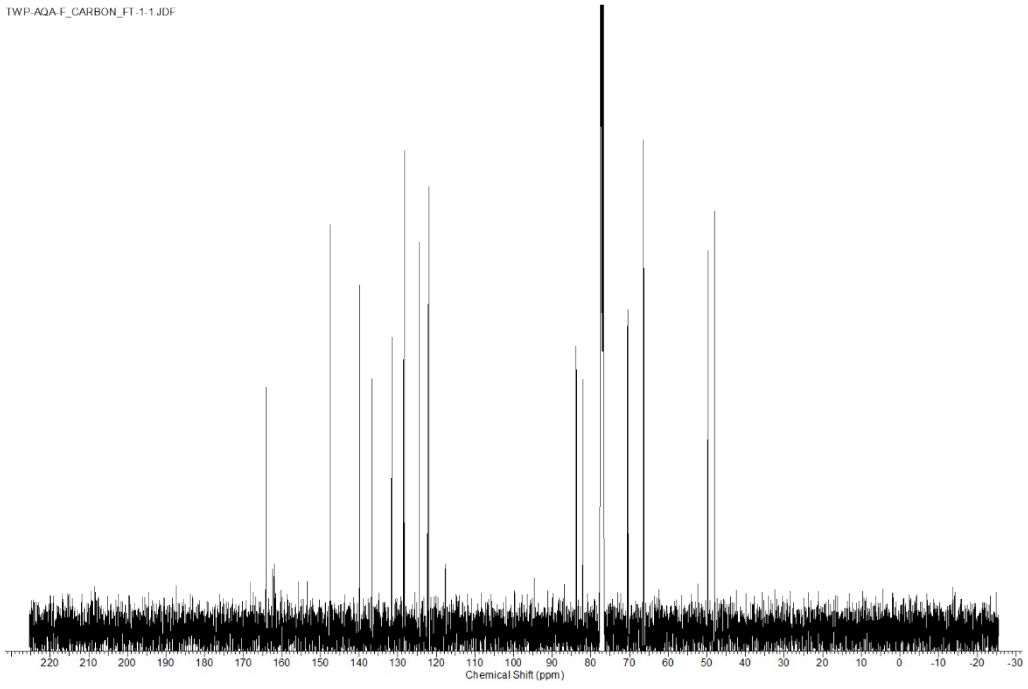


Figure S 8: ^{13}C NMR of **AQA-F** (5 mg ml^{-1})

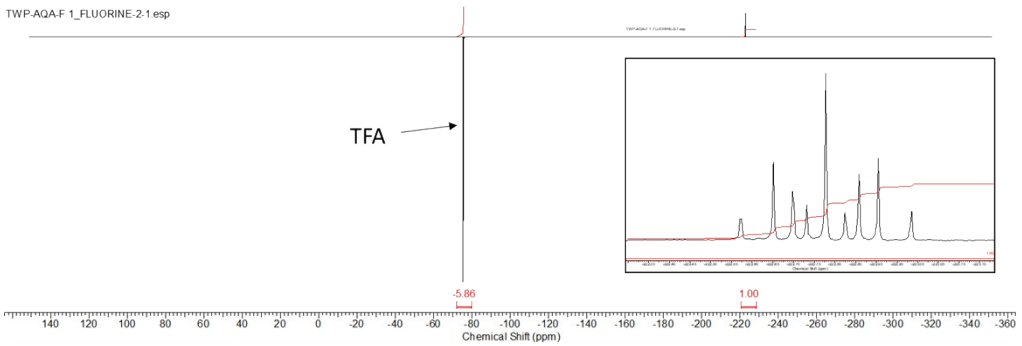


Figure S 9: ^{19}F NMR of **AQA-F**. Referenced to TFA. Inset: Splitting pattern observed for **AQA-F**. (5 mg ml^{-1})

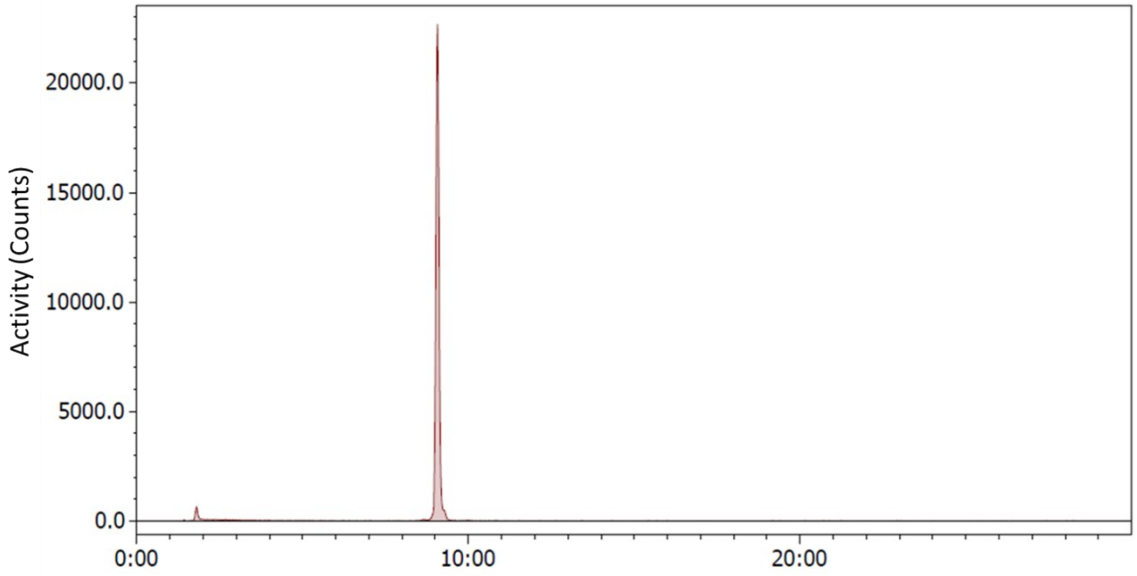


Figure S 10: Radio-HPLC chromatogram for purified [^{18}F]**AQA-F**

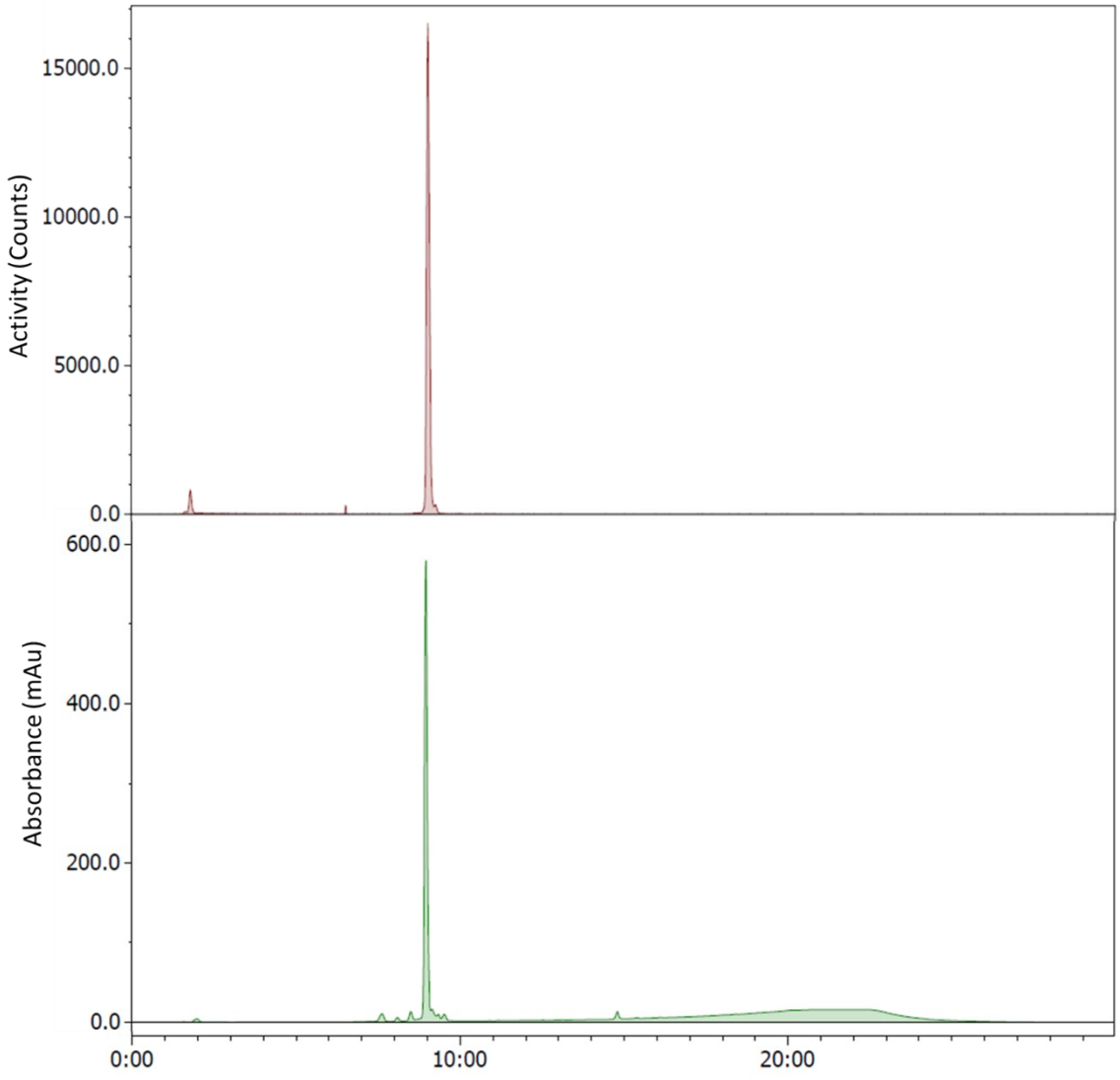


Figure S 11: HPLC chromatogram of purified [^{18}F]**AQA-F** coinjected with cold reference **AQA-F**

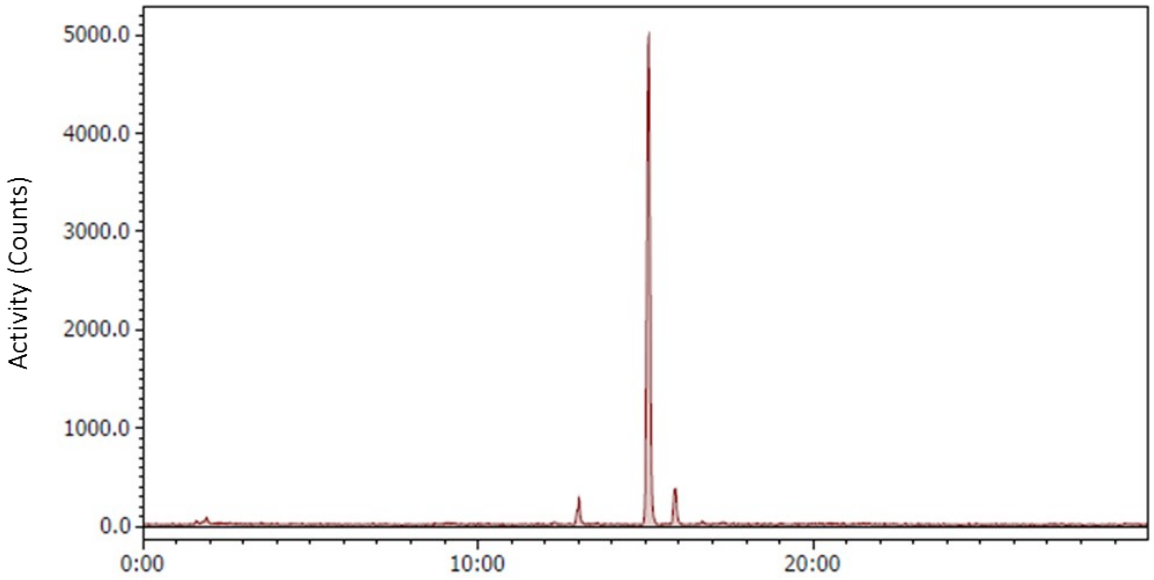


Figure S 12: Radio-HPLC chromatogram for crude radiolabelling mixture of **8** incubated with $[^{18}F]F^-$ prior to addition of TFA radiochemical conversion = 82.6%

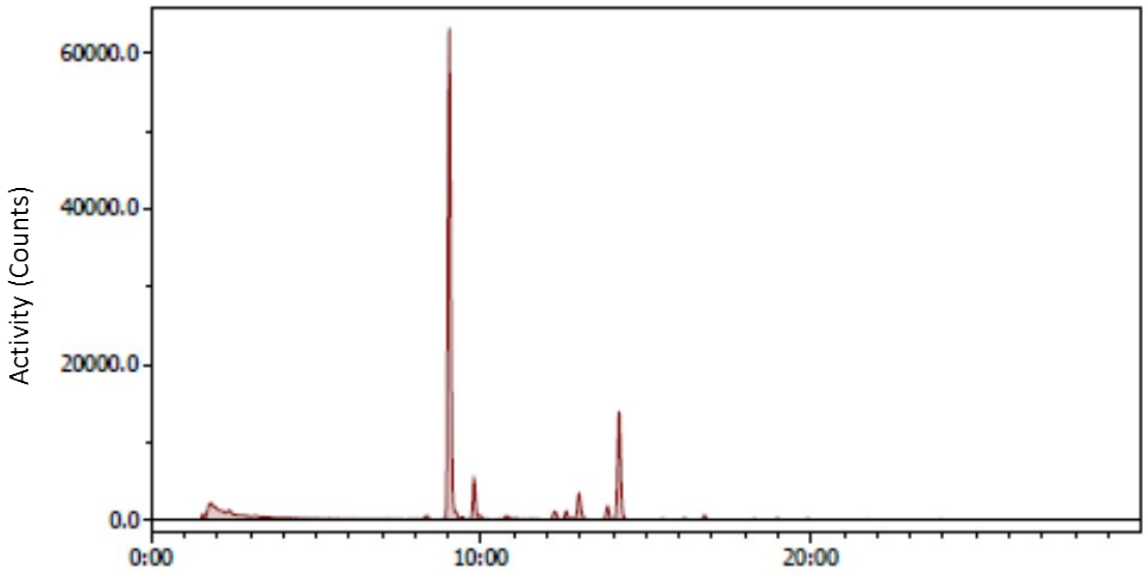


Figure S 13: Radio-HPLC chromatogram for crude radiolabelling mixture of **8** incubated with $[^{18}F]F^-$ 15 minutes after addition of TFA radiochemical conversion = 54.3%